酸化還元酵素であるグルコースオキシダーゼを固定化する。こうして得られたカーボン、クロルアニル、固定化グルコースオキシダーゼの混合物の少量とカーボン粉末をプレス成型により一体成型する。

上記の酵素電極を用いて、グルコース濃度を2×10^{-4}モル/ℓとしたときの電流値の変化を第4図に示す。またグルコース濃度と電流増加量の関係を第5図に示す。図より明らかなごとく、この酵素電極は、基質の添加に対して迅速に応答し、かつ残余電流も少なく、基質濃度変化に対する応答直線性も良好であるなど、優れた特性を有する。

酵素が、アルコール脱水素酵素などのように、補酵素を必要とする場合には、酵素とともに補酵素をも、電子伝導性物質と不溶性レドックス化合物の混合物上に固定化しておくと、前記同様に良好な応答特性が得られた。

レドックス化合物としては、クロルアニルの他にブロムアニルあるいは各種レドックスポリマーなどの不溶性レドックス化合物を用いても良い。

以上述べた如く、本発明によれば、酵素、レドックス化合物の有効利用を図り、きわめて容易に優れた性能を有する酵素電極を得ることができる。

4、図面の簡単な説明

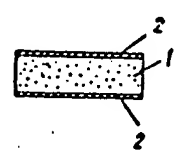
第1図は本発明の酵素電極の構成例を示す図、第2図は他の構成例を示す図、第3図は基質濃度の測定系を示す図、第4図は酵素電極のグルコースに対する応答特性を示す図、第5図はグルコース濃度と電流増加量との関係を示す。

1……第1の層、2……第2の層。

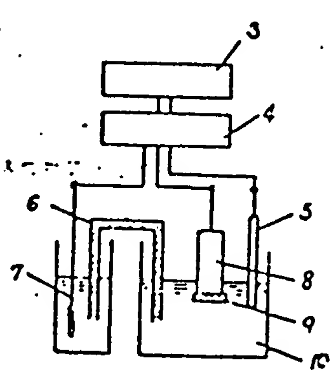
代理人の氏名　弁理士　中尾敏男　ほか1名

第1図

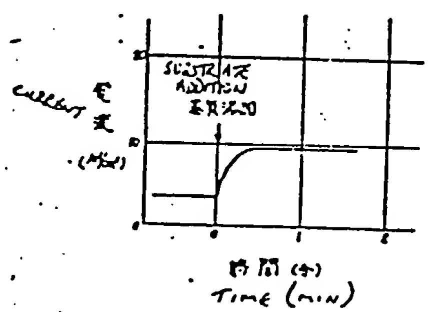
第2図



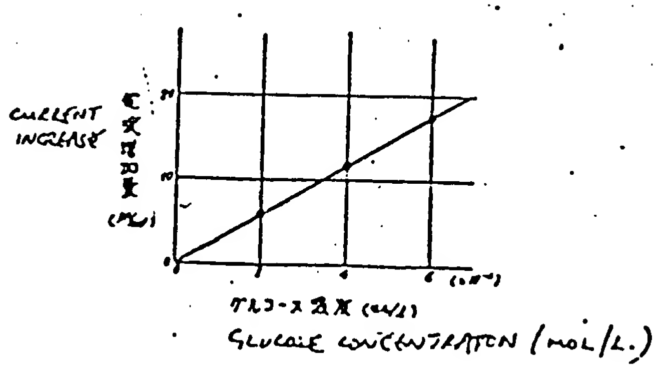
第3図



第4図



第5図



Japanese Patent 80 - 10584 25/1/80

Application 78 - 84482 10/7/78

Specification

1. Name of Invention

Enzyme Electrode and its Method of Production

2. Scope of Claim

1) An enzyme electrode characterised in that it has a layer 1 consisting of an electron conductive substance, and a layer 2 containing an electron conductive substance with an immobilised oxidising-reducing enzyme and an insoluble redox compound conjugate with the above-recorded enzyme.

2) An enzyme electrode as in 1) of the Claim where layer 2 fixes a co-enzyme of the above-recorded enzyme.

3) A method of production of an enzyme electrode characterised in that a layer consisting of an electron conductive material is moulded into one body with a layer wherein an oxidising-reducing enzyme is immobilised on a mixture of an electron conductive substance and an insoluble redox compound.

3. Detailed Specification

This invention intends to provide an enzyme electrode which has electrochemical activity against the substrate on which the specific catalytic activity of the enzyme operates, which enables rapid and simple measurement of the substrate concentration, and which is capable of continuous and repeated use. This invention relates to an enzyme electrode which, by its combination with an oxygen electrode, etc. may be used to convert the chemical energy of a substrate into electrical energy.

As an example of attempts at engineering applications of the specific catalytic action of an enzyme, trials have been carried out using a combination of an enzyme reaction system and an electrochemical reaction system to detect the concentration of a substrate which is the substance which reacts specifically with the enzyme. In treating an enzyme reaction as an electrochemical reaction, the method has been employed, for example,

where a suitable redox compound is introduced conjugately into an enzyme reaction system and where the oxidation-reduction reaction of this redox compound is detected electrochemically. In concrete terms, the redox compound which has been reduced (or oxidised) in the conjugate reaction with the enzyme is oxidised (reduced) electrochemically and the substrate concentration at this time can be detected as a current flow. However, because the expensive enzyme and redox compound are used in the dissolved state, they are lost each time a measurement is made, and the operation of the measurement itself is troublesome. In order to resolve these difficulties and facilitate repeated use of the enzyme, etc. and produce a practicable enzyme electrode, it is necessary that the enzyme and the redox compound be fixed into one body with an electron conductive substance as a collector.

As a result of various tests carried out by the inventors on methods to obtain an enzyme electrode where these enzymes and redox compounds are fixed into one body, they discovered the method where, for example, a mixture of carbon powder and an insoluble redox compound was press-moulded and an enzyme immobilised on this moulding; or the carbon powder on to which the enzyme had been immobilised beforehand was mixed into the above-recorded mixture and subsequently formed into a moulding. The enzyme electrodes thus obtained enabled rapid and convenient measurement of the substrate concentration.

As an improvement of the enzyme electrode, this invention, by means of its construction from a layer 1 consisting of an electron conductive substance and a layer 2 consisting of the electron conductive substance with the immobilised enzyme and a conjugate insoluble redox compound, largely reduces the amounts of enzyme and redox compound employed, and has succeeded in providing a high performance enzyme electrode. By means of this construction, enzyme electrodes can be obtained by a very convenient method of production.

Fig. 1 shows an example of enzyme electrode construction according to this invention. In the figure, 1 is the layer 1 consisting of the electron conductive material, 2 is the layer 2 consisting of the immobilised enzyme, the insoluble redox compound conjugate with the enzyme and the electron conductive substance, both of these layers being formed by moulding into one body. Layer 2 is the part which allows reaction between the substrate and the enzyme and redox compound, while layer 1 fulfils the roles of collector

and base for layer 2. As Fig. 2 indicates, these two layers may be combined so that the reaction layer is prepared on both sides, as necessary. Thus it is possible to construct an electrode with the minimum necessary amounts of enzyme and insoluble redox compound.

Next follows a description of a method of measurement using an enzyme electrode. Fig. 3 shows a measurement system for the measurement of a substrate concentration using an enzyme electrode according to this invention. In the figure, 3 is the recorder, 4 is the potentiostat, 5 is the reference electrode, 6 is the salt bridge, 7 is the opposite electrode, 8 is the holder to which is fixed the above-recorded enzyme electrode 9, 10 is the phosphoric acid buffer solution of pH 5.6 containing the substrate.

The enzyme electrode 9 is attached to the holder 8 so that layer 2 is in contact with the buffer solution, while to layer 1 is attached a lead of, for example, platinum.

After the enzyme electrode 9 has been immersed in the buffer solution, the electrode potential is maintained at a fixed level against the reference electrode, and the variations in the oxidation-reduction current of the redox compound which accompany changes in the substrate concentration are detected. At this time, even when there is no substrate present, a residual current flows which accompanies the oxidation-reduction of the electron conductive substance, or the sample enzyme used in the immobilisation, or impurities in the redox compound, etc. This residual current determines the S/N ratio of the response current produced by the reactions of the substrate, enzyme and redox compound. In order to raise the electrode performance, it is necessary that the redox compound and enzyme quantities be set at the minimum necessary limit, and that the optimum construction with the electron conductive material be used. By this means, design is possible for effective use of the high-priced enzyme and redox compound. Metals stable in oxidation-reduction, or conductive metal oxides such as tin oxide, etc. can be used as the electron conductive substance. In particular, carbon is a chemically stable and a good electrically conductive material; also it does not hinder the enzyme reaction, etc. and so is favourable as the electron conductive material.

Next follows a description of the method of production of an enzyme electrode. First the powdered electron conductive material and insoluble redox

compound are well mixed. On to this mixture the enzyme (and, if necessary, the co-enzyme) is immobilised using a cross-linking reagent such as glutaldehyde, etc. Next a small amount of the mixture obtained, consisting of the electron conductive material, the insoluble redox compound and the immobilised enzyme, and the electron conductive substance are formed into one moulding, for example by press-moulding. A binder may also be used to increase the strength of the moulding. As well as the method recorded above, various other types of method can be used in the immobilisation of the enzyme, such as direct chemical coupling of the electron conductive substance and the enzyme.

Below is a description of this invention given by the example of an embodiment.

Carbon powder, such as acetylene black, graphite, etc. as the electron conductive material, is well mixed with chloranil as the insoluble redox compound. Next the reduction-oxidation enzyme glucose oxidase is immobilised on this mixture using glutaldehyde. A small amount of this mixture of carbon, chloranil, and immobilised glucose oxidase is press-moulded into one body together with some carbon powder.

Fig. 4 shows the variations in current values when using the above-described enzyme electrode, the glucose concentration was set at 2 x 10^{-4} mol/litre. Fig. 5 shows the relationship between glucose concentration and current increase. As is clear from the figures, this enzyme electrode has the excellent characteristics that it responds rapidly to the addition of substrate, it has a low residual current, and it has favourable response linearity against substrate concentration changes.

Where the enzyme requires a co-enzyme, as in the case of alcohol dehydrogenase, and when the enzyme together with the co-enzyme is immobilised on the mixture of electron conductive substance and insoluble redox compound, favourable response characteristics similar to those above are obtained.

As well as chloranil, other insoluble redox compounds such as bromanil, or redox polymers, may be used as the redox compound.

As has been related above, by this invention it is possible to obtain very easily an enzyme electrode with excellent performance, and which is designed for effective utilization of the enzyme and redox compound.

4. Simple Description of Figures

Fig. 1 shows an example of the construction of an enzyme electrode of this invention; Fig. 2 shows another example of construction; Fig. 3 shows the system of measurement of substrate concentration; Fig. 4 shows the response characteristics of the enzyme electrode against glucose; and Fig. 5 shows the relationship between glucose concentration and current increase.

1 . . . . . layer 1

2 . . . . . layer 2

Name of Agent, Nakao Toshio, Attorney-at-Law and one other.